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Fig.2.

gcagatgcccgaagctttttatagcgg HindIII

aroB1-

melting temperature=74°C

gagctcggtaccgtgcagcgtgccagatctgcaag

aroB2-

melting temperature=72°C

Sad KpnI

melting temperature=70°C

cataaagggatccgtgttcgccagc

aroB3-

BamH

melting temperature=74°C

Kpnl Sad

ggtaccgagctccaaatgaaggcagatctcgtcgccc

aroB4-

aroB1 + aroB2 - gives a fragment of 1575 bp. From upstream of aroB.

Amplify the two halves of the aroB region by PCR using the above primers in the following combinations

aroB4 + aroB3 - gives a fragment of 1433 bp. From downstream of aroB.

Ligation of these fragments together gives a deletion in the middle of aroB of approximately 150 bp.

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3/11 띮 BamHI Cut with *Bam*HI and *Kpn*I then cloned into pGIT5 pGIT5aroB3-4 Kanamycin R →—BamHI aroB3 Bg/Ⅱ Kpnl∢ HindⅢ、 pCR3-4 Cut with Hind and Kpnl. Then ligated with Hind and Kpnl cut pCR1-2 aroB4 Fig.3(i) Kpnl Saci Bg/II deletion aroB2 Bg/II Kpnl Sacl aroB pCR1-2 CIT CIT BamHI Hind aroB1 pCR1-2 cut with Hind回 and KpnI / pGIT5aroB∆1-4 Kanamycin R Bg/Ⅲ Bg/Ⅲ Konl

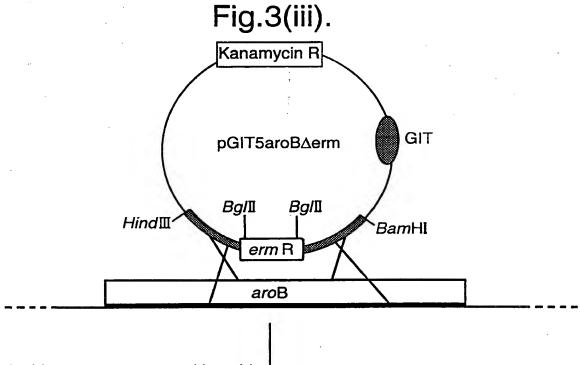
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Hind

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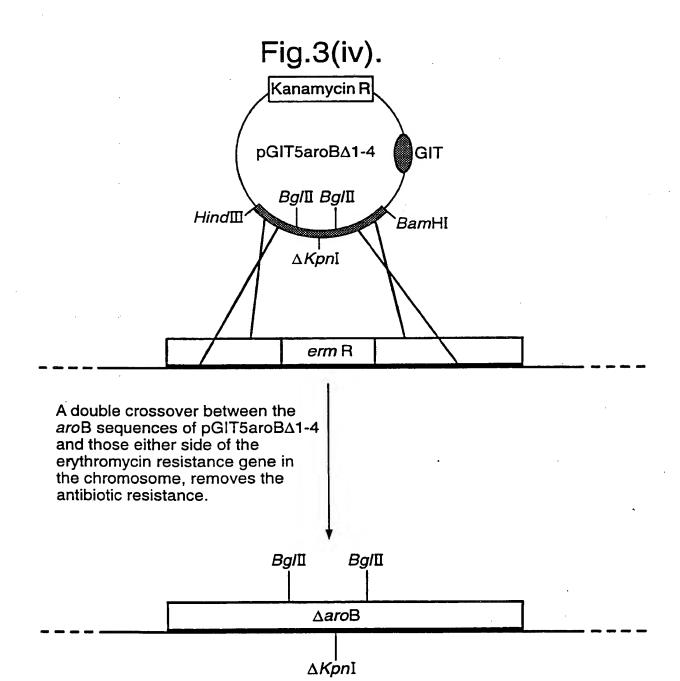


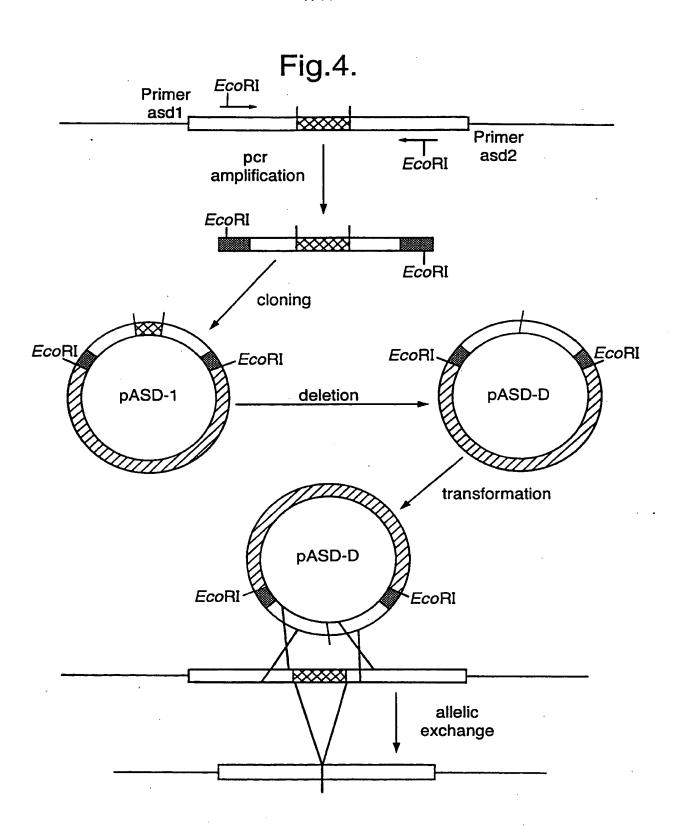
A double crossover event either side of the erythromycin resistance gene replaces the wild type gene with the deletion derivative containing the erythromycin resistance gene

erm R

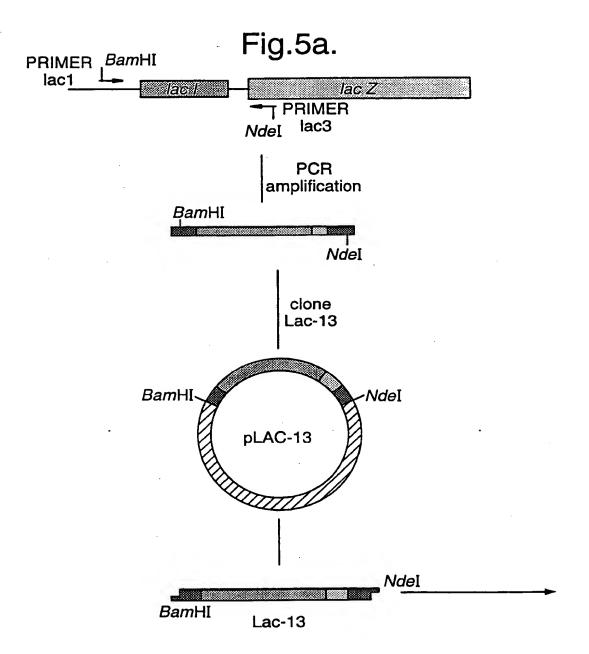
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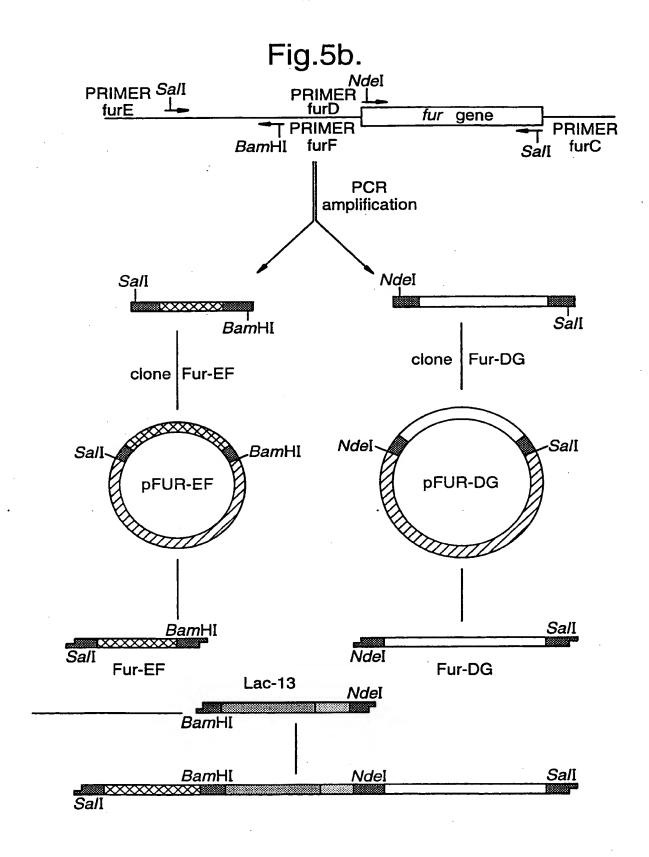
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